# Ultrasonic Vibration Effects on the Development and Respiratory Metabolism of the Egg of *Tenebrio molitor* Linn. (Coleoptera: Tenebrionidae)[1]

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**Abstract:** When eggs of the mealworm, *Tenebrio molitor*, were allowed to develop at 30° C., 85.1% of them hatched. Treatment of eggs at different stages of development with ultrasonic vibrations of 20 kilocycles per second for 15 seconds at 10, 20, and 30 watts resulted in values ranging from 1.8 to 46.8%. Earlier stages were more sensitive than the later ones. Correspondingly, the rate of oxygen consumption was reduced more during early development than at later stages. There was a significant decrease during the first four days, whereas during the last two days the decreases were not significant. These effects may be correlated with the embryological events occurring within the eggs at the time of their treatment and with the structure of the egg membrane.

Ultrasonic waves (high-frequency sound waves above the upper limit of human hearing) have been found to influence development in a variety of ways, ranging from no effect to those which are lethal. Effects include acceleration of development, parthenogenetic stimulation of development, acceleration of metabolism, an increase in growth and size, inhibition of growth, depressed development, reduction in size, delayed maturation, modification in the percentage of survival, abnormalities, and mutations.

Treatment of eggs of wild type *Drosophila virilis* with ultrasonic vibrations of 450 kilocycles per second (kc./sec.) by Takebe (1944) resulted in 22% "vestigial"-winged individuals that were shown to be a phenocopy. Fritz-Niggli and Böni (1950) and Fritz-Niggli (1951) treated eggs of a wild stock of *D. melanogaster* with vibrations of varying intensities from 0.3 to 4.0 watts/cm$^2$ and found that the radiation produced a delayed reaction, with death sometimes occurring in later stages. When the adults derived from treated eggs were examined, damage was found but no lethal mutations were noted. Counce and Selman (1955) reported different types of abnormalities in embryonic development after treatment of eggs of *D. melanogaster* with low intensity vibrations of 1000 kc./sec. for 30

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seconds. Sautet, Levavasseur, and Vuillet (1947) exposed eggs of mosquitoes to vibrations of 960 kc./sec. and observed that the eggs of *Anopheles maculipennis* and *Culex pipiens* were more sensitive to ultrasound than those of *Aedes mariae*. At high intensities, for a 30 second exposure, eggs burst readily, while at low intensities there was no destruction, but a stimulation and acceleration of hatching. Eggs of the yellow-fever mosquito, *Aedes aegypti*, treated for 15, 20, 25, and 30 seconds with waves of 500 kc./sec. by Quraishi, Osmani, and Ahmad (1963) showed percentages of hatching of 71.2, 68, 64.6, and 55, respectively, as compared with 62.5 in the control. Getsova, Lapinskaia, and Khenokh (1958) observed that exposure of the eggs of the Shantung silkworm, *Antheraea pernyi*, to vibrations of 125 kc./sec. at 300 to 400 watts for 1 to 30 minutes, at different periods from the beginning of development through formation of the "primitive groove" had a stimulating effect and increased the embryonic development rate. Subjection of the egg to ultrasound during the stage of blastokinesis, when the embryo is already formed, had no stimulating effect, and led to the death of the embryo.

The effects of ultrasound on *Tenebrio molitor* have been investigated on larvae and pupae. Frings, Allen and Rudnick (1948) found that the larvae were killed in 30 seconds when exposed to high-intensity air-borne ultrasonic waves of 18.5–19.0 kc./sec. Kocian (1936) observed that short exposures of 30 seconds, and 1 minute to a high-frequency electric field had no effect on respiration or metamorphosis of pupae, but exposures of 5–10 minutes shortened the pupal period and lowered oxygen consumption. Kadoum, Nelson, and Stetson (1967) found that the mortality of larvae exposed to radiofrequency electric fields of 39,000 kc./sec. increased with exposure time and intensity. Kadoum, Ball, and Nelson (1967) observed that adults developing from larvae exposed at sublethal levels to this same frequency had malformed and missing legs. Kadoum, Ball, and Stetson (1967) reported that when larvae were briefly treated under the same conditions, a progressive loss of body weight occurred during the ensuing week. Younger larvae lost proportionately more weight than older ones. The present investigation was conducted to obtain information about the response of the egg of *Tenebrio molitor* to vibrations of 20 kc./sec. and the influence of ultrasound on the physiology of development.

## MATERIALS AND METHODS

Pupae of the mealworm, *Tenebrio molitor*, were collected from our laboratory stock cultures, placed in culture dishes and kept in an incubator at 30° C. Newly emerged adults were transferred to culture dishes containing chick growing mash and kept at 30° C. for the preoviposition period of four days. Moisture was supplied by cloth covers to which tap water was added several times a week. To eliminate any possible parental age effects, as found by Ludwig and Barsa (1955), Ludwig (1956), Tracey (1958), Ludwig and Fiore (1960, 1961), and

Ludwig, Fiore and Jones (1962), only mature young adults of 2 to 4 weeks were used in these experiments. For egg collection, they were placed in culture dishes containing white flour. A vial of water with a moist cotton plug in each culture dish was the water source. Eggs were collected each day by sifting the flour. They were kept in dated vials and placed in a desiccator, the base of which contained a saturated solution of NaCl (relative humidity 75%) and incubated at 30° C. The age of each egg was known to within 24 hours.

Eggs for each day of embryonic development at 30° C. from the day of collection (0 day) to just before hatching (5 day) were used to study the effects of ultrasonic vibrations on the percentage of hatching and the rate of oxygen consumption.

The Bronwill-Blackstone Biosonik Needle Probe was used as the source of ultrasonic vibrations. Groups of 50 eggs for each day of embryonic development were weighed and placed in 18 ml. shell vials. Immediately before treatment, 7 ml. of distilled water was added to the eggs in the vial, after which the tip of the vibrating needle probe was lowered 7 mm. into the distilled water.

Preliminary experiments were run for periods of 15, 30, 45, and 60 seconds, with vibrations of 20 kc./sec. at a power setting of 10 watts to determine the optimal time of treatment. As a result, 15 seconds was chosen as a sublethal dose and eggs were treated for 15 seconds at power settings of 10, 20, and 30 watts (approximate intensities of 0.178 watts/cm$^2$, 0.356 watts/cm$^2$, and 0.534 watts/cm$^2$, respectively). The temperature of the distilled water was checked during treatment. Since ultrasonic treatment was limited to short periods of time, the experimental temperature remained below 30° C. The treated eggs were removed immediately from the distilled water, blotted dry with filter paper, placed in stender dishes, and incubated at 30° C. Control groups were run under the same conditions without ultrasonic treatment. The duration of the egg stage and the percentage of hatching of both groups (treated and control) were determined and compared.

To study the effect of ultrasonic vibrations on the respiratory metabolism of the egg, measurements of oxygen consumption were made on both control and treated eggs in groups of 50 at ages of 0, 1, 2, 3, 4, and 5 days. Bronwill Differential Respirometers were used at 30° C. following the procedure outlined by Umbreit, Burris and Stauffer (1957).

## OBSERVATIONS

The percentage of hatching in 700 untreated eggs averaged 85.1%. In treated eggs, as shown in Table 1, it ranged from 1.8 to 46.8% regardless of the intensities used. Resistance to ultrasonic vibrations increased with the age of the egg at the time of treatment.

No observable change in the time of hatching was noted for eggs treated with ultrasonic vibrations when compared with normal eggs. Treated eggs allowed

Table 1. Percentages of hatching of ultrasonicated eggs of the mealworm, *T. molitor*, treated during embryonic development for 15 seconds with ultrasonic vibrations of 20 kilocycles per second at power settings of 10 watts, 20 watts, and 30 watts.

| Age of eggs | Number of eggs used | % hatched with 20 kc./sec. 15 sec., 10 watts | % hatched with 20 kc./sec. 15 sec., 20 watts | % hatched with 20 kc./sec. 15 sec., 30 watts |
|---|---|---|---|---|
| 0 day | 1550 | 1.8 | 1.8 | 1.8 |
| 1 day | 2050 | 4.6 | 2.5 | 1.2 |
| 2 day | 1800 | 9.1 | 6.0 | 7.6 |
| 3 day | 1550 | 31.4 | 25.8 | 23.2 |
| 4 day | 1900 | 36.0 | 35.7 | 40.9 |
| 5 day | 2350 | 46.8 | 46.7 | 46.7 |

to hatch produced normal larvae, pupae, and adults. There was no observable difference in the gross morphology of these individuals when compared to those that were untreated.

The effects of ultrasonic vibrations on oxygen consumption are shown in Table 2. The rate of oxygen consumption was considerably lower in treated eggs than in the controls. Percentage values were calculated, and the treated 0, 1, 2, and 3 day eggs showed a reduction in oxygen consumption ranging from 12.9 to 28.5% with most values representing a reduction of 20% or more. Eggs treated on the last two days of embryonic development exhibited a reduction in oxygen consumption ranging from 8.4 to 22.6% with most values representing a reduction of less than 20%.

A statistical treatment of the values obtained for oxygen consumption in normal and treated eggs is given in Table 3. Significant decreases were observed in all instances when 0, 1, 2, and 3 day eggs were treated at power settings of 20 and 30 watts. At 10 watts the decrease was not significant. Treatment of 4 and 5 day eggs at 10 and 20 watts did not result in significant decreases, but at 30 watts there was a significant reduction in 5 day eggs.

Table 2. Comparison of oxygen consumption in untreated and ultrasonicated eggs of the mealworm, *T. molitor*, treated during embryonic development for 15 seconds with ultrasonic vibrations of 20 kilocycles per second at power settings of 10 watts, 20 watts, and 30 watts. The rate of oxygen consumption is expressed as microliters of oxygen/50 eggs/hour. Values are given with their standard errors.

| | Microliters oxygen/50 eggs/hour | | | |
|---|---|---|---|---|
| Age of eggs | Untreated eggs | 20 kc./sec. 15 sec., 10 watts | 20 kc./sec. 15 sec., 20 watts | 20 kc./sec. 15 sec., 30 watts |
| 0 day | $8.17 \pm 0.55$ | $6.82 \pm 0.69$ | $6.37 \pm 0.43$ | $5.98 \pm 0.73$ |
| 1 day | $11.42 \pm 1.05$ | $9.77 \pm 0.99$ | $8.63 \pm 0.85$ | $8.77 \pm 0.56$ |
| 2 day | $11.36 \pm 0.87$ | $8.85 \pm 0.84$ | $9.17 \pm 0.57$ | $8.12 \pm 0.76$ |
| 3 day | $11.20 \pm 0.74$ | $9.76 \pm 0.71$ | $8.95 \pm 0.44$ | $8.77 \pm 0.81$ |
| 4 day | $14.21 \pm 1.39$ | $12.46 \pm 1.10$ | $12.50 \pm 1.25$ | $11.39 \pm 0.83$ |
| 5 day | $16.18 \pm 1.11$ | $14.38 \pm 1.38$ | $14.82 \pm 1.29$ | $12.53 \pm 0.80$ |

TABLE 3. Probable significance of differences obtained in the rates of oxygen consumption in untreated eggs of the mealworm, *T. molitor*, and eggs treated during embryonic development for 15 seconds with ultrasonic vibrations of 20 kc./sec. at power settings of 10, 20, and 30 watts.

| Ages compared | Difference between means | Standard error of difference | P* value |
|---|---|---|---|
| *0 day* | | | |
| Untreated and treated at 10 watts | 1.35 | ± 0.88 | > 0.1 |
| Untreated and treated at 20 watts | 1.80 | ± 0.70 | < 0.02 |
| Untreated and treated at 30 watts | 2.19 | ± 0.91 | < 0.05 |
| *1 day* | | | |
| Untreated and treated at 10 watts | 1.65 | ± 1.44 | > 0.2 |
| Untreated and treated at 20 watts | 2.79 | ± 1.35 | 0.05 |
| Untreated and treated at 30 watts | 2.65 | ± 1.19 | < 0.05 |
| *2 day* | | | |
| Untreated and treated at 10 watts | 2.51 | ± 1.21 | < 0.05 |
| Untreated and treated at 20 watts | 2.19 | ± 1.04 | < 0.05 |
| Untreated and treated at 30 watts | 3.24 | ± 1.16 | 0.01 |
| *3 day* | | | |
| Untreated and treated at 10 watts | 1.44 | ± 1.03 | > 0.1 |
| Untreated and treated at 20 watts | 2.25 | ± 0.86 | < 0.02 |
| Untreated and treated at 30 watts | 2.43 | ± 1.10 | < 0.05 |
| *4 day* | | | |
| Untreated and treated at 10 watts | 1.75 | ± 1.77 | > 0.3 |
| Untreated and treated at 20 watts | 1.71 | ± 1.87 | > 0.3 |
| Untreated and treated at 30 watts | 2.82 | ± 1.62 | > 0.05 |
| *5 day* | | | |
| Untreated and treated at 10 watts | 1.80 | ± 1.77 | > 0.3 |
| Untreated and treated at 20 watts | 1.36 | ± 1.70 | > 0.4 |
| Untreated and treated at 30 watts | 3.65 | ± 1.37 | < 0.02 |

* Probability values of 0.05 or less are considered statistically significant.

## DISCUSSION

Embryonic development in the egg of *Tenebrio molitor* has been described by Ewest (1937) as consisting of blastoderm formation, stratification, separation of organ systems, and histological differentiation. The extreme sensitivity of 0 day eggs to ultrasound may be associated with the processes of cleavage and blastoderm formation occurring at this time. Since eggs were collected every 24 hours, those labelled 0 day may vary in age from just laid to 24 hours. During this period the observation of the following has been reported: maturation divisions and union of the male and female pronuclei; cleavage divisions and population of the egg by 512 nuclei; separation of primary and secondary "vitellophagen" or yolk liquifying cells; migration of nuclei towards the periphery of the egg and their entrance into the peripheral layer to form the blastoderm. This is in agreement with Counce and Selman (1955) who noted that treatment at any particular ultrasonic intensity for a given time produced maximum lethality when the eggs of *Drosophila melanogaster* were at the stage during which the nuclei migrate to the periphery to form the blastoderm. The continued sensitivity of 1, 2, and 3 day eggs to ultrasonic vibrations, although not as marked as that of 0 day eggs, may be associated with the formation and growth of the germ band and the separation of the organ systems taking place during this period. The greater resistance of the 4, and especially the 5 day eggs, to ultrasound may be associated with histological differentiation. During this time dorsal closure, pigmentation of eyes and mouth extremities, completion of the embryo, active movement and hatching occur. According to Fritz-Niggli and Böni (1950), it seems probable that early egg stages of an insect are more susceptible to ultrasonic vibrations than are later stages which are characterized by proliferations or cytological differentiation of tissues.

That resistance to ultrasound increases with age is supported by the data obtained both for the percentage of hatching and the rate of oxygen consumption for eggs treated on each of the days of embryonic development in the present work, as well as by reported results of other investigators. Fritz-Niggli and Böni (1950) found that in *D. melanogaster* resistance to ultrasonic intensities of 0.3 to 4.0 watts/cm$^2$ at the beginning of puparium formation increased nearly a thousandfold as compared with that of the egg when laid.

Increased resistance of the egg to ultrasound with age may also be associated with the structure of the egg membranes. Ewest (1937) observed that the egg of *T. molitor* is covered by an inner delicate vitelline membrane and an outer coarse chorion. These coverings are soft and sticky in the freshly laid egg but strengthen during the developmental period.

The reduction in the percentage of hatching from 85.1% in the normal egg of *T. molitor* to values ranging from 1.8% after ultrasonic treatment of 0 day eggs to 46.8% in treated 5 day eggs is much greater than that obtained by Quraishi, Osmani, and Ahmad (1963) when eggs of the mosquito, *Aedes aegypti*, were treated with vibrations of 500 kc./sec. for 30 seconds. They found a reduction from 62.5 to 55%. These same investigators found that when the eggs were treated for 25 seconds, the percentage of hatching was equivalent to the control; and after treatment for 15 and 20 seconds, a higher percentage resulted. A stimulation and acceleration of hatching was reported by Getsova, Lapinskaia, and Khenokh (1958) for eggs of the Shantung silkworm, *Antheraea pernyi*, treated for 1 to 30 minutes with waves of 125 kc./sec., and by Sautet, Levavasseur, and Vuillet (1947) for eggs of the mosquitoes, *Anopheles maculipennis*, *Culex pipiens*, and *Aedes mariae* treated for 30 seconds with vibrations of 960 kc./sec. These variations in the influence of ultrasound on the percentage of hatching may be explained by the observations of many investigators that the effects depend on the characteristic features of different species as well as on the intensity and duration of treatment.

The significant decrease in the rate of oxygen consumption observed in 0, 1, 2, and 3 day eggs of *T. molitor* after ultrasonic treatment may be compared with the results obtained by Kocian (1936), who found that exposure of 5 to 10 minutes in a high frequency electric field lowered the oxygen consumption in pupae of *T. molitor*. Kadoum, Ball, and Stetson (1967) found that the rate of oxygen consumption rose promptly when larvae of *T. molitor* were exposed to radiofrequency electric fields of 39,000 kc./sec.

The lower rate of oxygen consumption in eggs of *T. molitor* subjected to ultrasound suggests an altering of tissue metabolism and an adverse effect on the overall physiological state of the developing embryo. Enzyme disturbance may be responsible for the resulting metabolic changes. According to Él'piner (1964) and Él'piner, Faikin, and Basurmanova (1965) nonlethal doses of ultrasound may bring about the appearance of delicate, possibly reversible, biochemical and functional changes in the irradiated organisms. In these conditions not only are the processes of permeability and diffusion in the superficial layers of the cell modified, but also spatial relationships between the submicroscopic structures and between the macromolecular complexes incorporated in these structures are disturbed. These authors postulate that as a result of these disturbances, the spatial orientation of the cellular macromolecules is modified, and is accompanied by the "exposure" of new enzymic centers, functional groups of molecules, and specific receptors, situated mainly in the superficial layers of the cell.

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